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The Nature of Cytoplasmic Inclusions in Cerebellar Haemangioblastomas

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Summary. Intracytoplasmic hyaline globules present in stromal cells in eight of a series of ten cerebellar haemangioblastomas have been shown to contain the glycoprotein alpha-1-antitrypsin.

Key words: Cerebellar haemangioblastomas – Hyaline globules – Alpha-1-antitrypsin

Introduction

Globular eosinophilic inclusions have been observed in the cytoplasm of tumour cells in cerebellar haemangioblastomas. It was noted that they were morphologically similar to the inclusions seen in the liver in alpha-1-antitrypsin (AAT) deficiency disease [2], as well as having identical staining reactions. The peroxidase-anti-peroxidase (PAP) technique using AAT antiserum has proved useful in determining the nature of these inclusions.

Material and Methods

A series of ten haemangioblastomas was examined. Nine were derived from the surgical pathology files of the Royal Free Hospital, London, and further post mortem case kindly provided by Dr. R. O. Barnard from Maida Vale Hospital for Nervous Diseases, London. There were three men, 33, 38 and 52 years, and seven women, their ages ranging from 41 to 67 (mean 56 years). All tumours came from the posterior cranial fossa.

Sections of formalin-fixed, paraffin-embedded material were cut at 3 μm and stained with haematoxylin-eosin (HE), PAS after amylase digestion, PTAH, phloxine-tartrazine and Lendrum's Picro-Mallory trichrome. Additional staining for AAT was carried out using the PAP method, the sections treated with 0.1% trypsin made up in 0.1% calcium chloride (pH 7.4, at 37° C for 15 min.). The antiserum was diluted 1/200, and 3,3′-diaminobenzidine tetrahydrochloride used as the chromagen. Endogenous peroxide was blocked with 1% hydrogen peroxide in methanol.

Positive control sections from liver in a known case of AAT deficiency disease were used. Negative controls for each case and for the control material were processed without exposure to AAT antiserum. Normal tonsil was also used as a reference for AAT staining pattern in normal histiocytes.

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Results

In eight of ten haemangioblastomas, eosinophilic globular inclusions were present in the cytoplasm of scattered stromal cells, and also apparently lying free in the extracellular space. Although usually poorly visualised in HE sections, the inclusions showed distinctive and consistent magenta staining with the amylase-PAS method, which facilitated low-power scanning of sections (Fig. 1). The globules stained orange to red with phloxine-tartrazine and the trichrome, and light purple to black with PTAH. Inclusions in the liver control showed identical staining reactions.

Globules were occasionally single, and in two cases found only after careful search. In the remaining six cases, however, clusters of inclusions of varying size were easily detected in several areas of the tumour. The diameter of the inclusions varied from less than 1 μm to 50 μm, and occasionally up to 100 μm; in the AAT deficiency control section the maximum diameter was about 10 μm.

Specific AAT immunoperoxidase reactivity was present in relation to both the globules in the control and in all eight test sections where globules had been identified previously. A characteristic ring pattern of positive staining, in which the centre of the inclusion was only lightly stained or remained unstained, was seen (Figs. 2, 3). Histiocytes in the tonsil control section showed a similar cytoplasmic pattern of positive staining for AAT. No globules were, however, identified in their cytoplasm with the other stains.

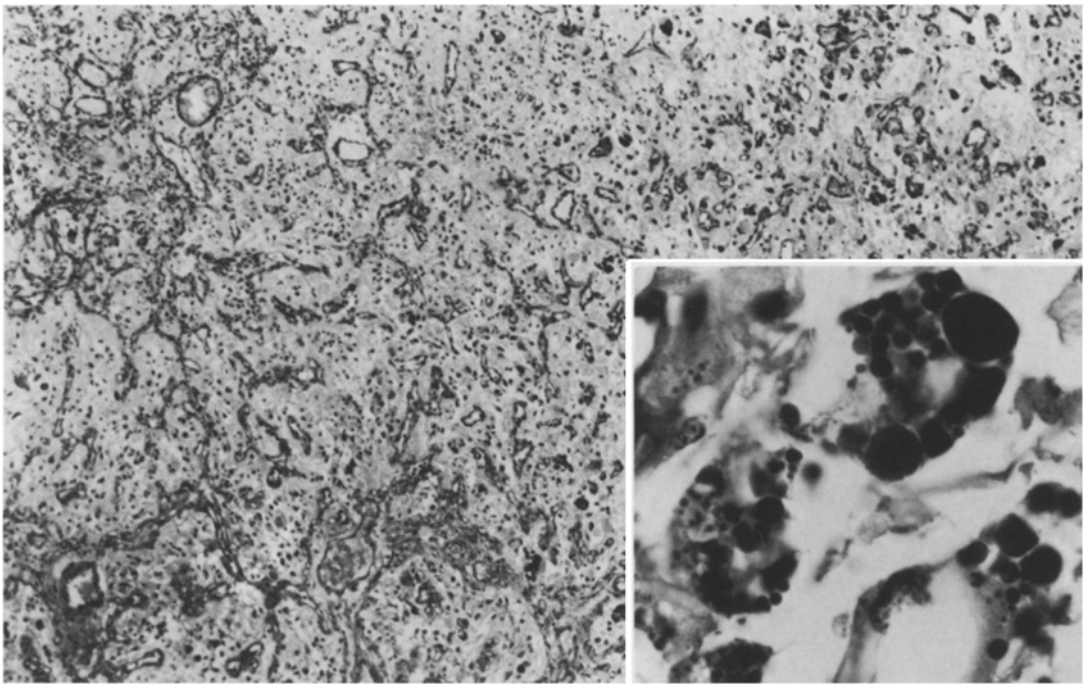

Fig. 1. Cerebellar haemangioblastoma. Spherical inclusions of varied diameter in the cytoplasm of stromal cells. PAS-D, ×75. Inset: PAS-D, ×630

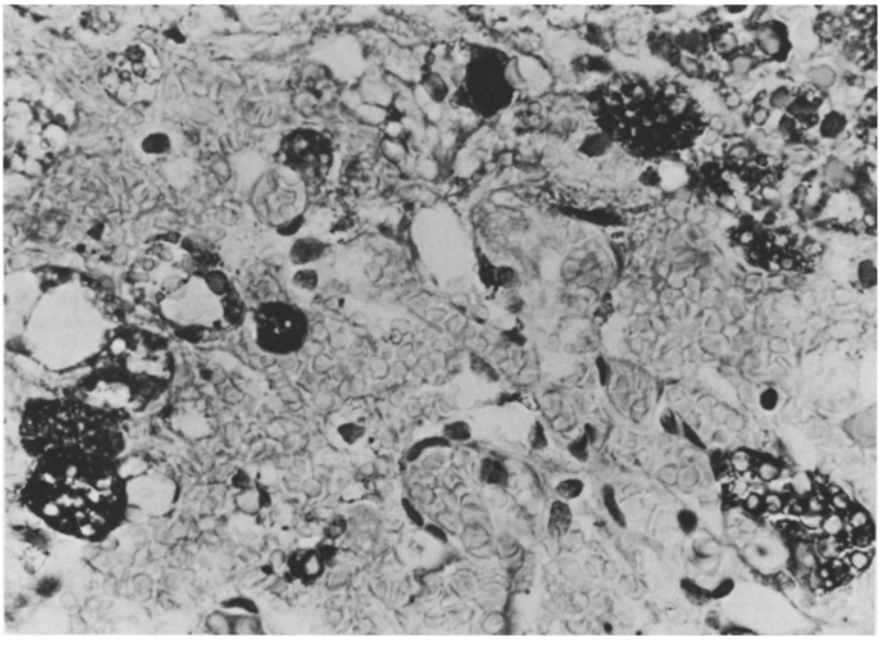

Fig. 2. Strongly positive staining for AAT shows typical ring-like granular precipitate of reaction products. AAT, ×630

Discussion

"Hyaline globules", reported before the advent of immunohistochemical techniques, have been observed in carcinomas of the lung, liver, breast and kidney [3–5], as well as in normal liver and adrenal medulla [2, 8]. Although they may represent a heterogeneous group of substances, AAT has recently been identified in many such globules, and the list of tissues and neoplasms showing positive staining for this glycoprotein has grown. Among others, cells of the macrophage series, hepatocytes, gut mucosal cells and pancreatic cells may all contain AAT [7, 11, 12]. Tumours in which AAT has been found include the apparently unrelated malignancies hepatocellular carcinoma, gastric carcinoid, signet-ring carcinoma of rectum, malignant mixed mesodermal tumours of the ovary, yolk-sac tumours, and anaplastic carcinoma of

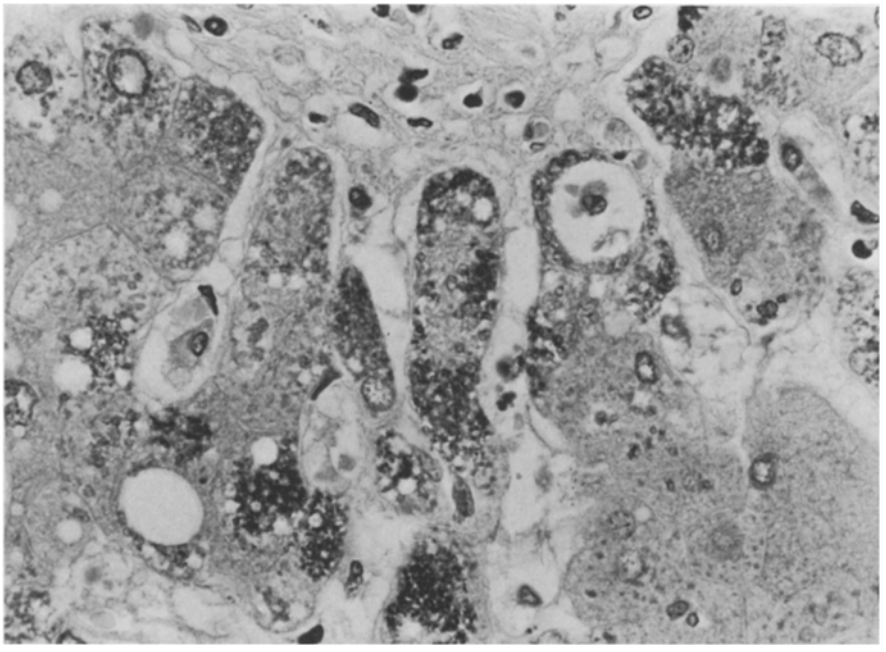

Fig. 3. Periportal cells in the liver of AAT deficiency disease control show similar globules. AAT, ×630

lung [1, 6, 9–12]. No tumour of the central nervous system (CNS) has yet been reported to contain AAT, so far as we are aware.

The inclusions seen in our series of haemangioblastomas and the AAT deficiency liver control are identical morphologically and in all staining reactions. In addition, a similar, though more granular, pattern of immunoperoxidase reactivity was observed in normal histiocytes in control sections. In view of these findings it is difficult not to accept that the inclusions we have seen in these tumours are composed of AAT itself, or at least some related protein.

The significance of finding AAT in a tumour is not known at present. It has been speculated that the presence of AAT in the neoplastic cells may represent primary synthesis of the protein by the tumour [10]. An alternative possibility is that the appearance is artefactual, resulting from phagocytosis or absorption of AAT by tumour cells from serum proteins [10, 12], especially in the presence of inflammation or necrosis; however, the majority of cases including those here recorded of cerebellar haemangioblastoma are entirely free of necrosis or inflammatory infiltration.

In AAT deficiency disease the inclusions have been localised ultrastructurally in the dilated endoplasmic reticulum of hepatocytes: this has not as yet been demonstrated in the stromal cells of the haemangioblastoma.

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